Identification, Pathogenicity, and Fungicide Sensitivity of *Pythium* spp. Associated with Corn and Soybean Seed and Seedling Disease in Ohio.

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Abstract

Cool moist conditions in combination with minimum tillage, earlier planting, and recent changes in commercial seed treatment active ingredients have led to an increase in corn and soybean stand establishment problems. Persistent reduction in stands over the years has led to the current investigation of *Pythium* species associated with seed and seedling disease of corn and soybean in Ohio. Samples of diseased corn and soybean were collected from 40 locations in Ohio and northeastern Indiana. All isolates of *Pythiu* recovered were identified to species, evaluated in an *in vitro* pathogenicity assay on both corn and soybean seeds, and a subset of the isolates were tested for their sensitivity to fungicides currently used as seed treatments. Twelve species and two distinct morphological groups of *Pythium* were identified, of which 6 species were moderately to highly pathogenic on corn seeds and 9 species were highly pathogenic on soybean seeds. There was significant variation ($P<0.01$) in sensitivity to mefenoxam, azoxystrobin, trifloxystrobin, and Captan both across species and within species of *Pythium*. This research has shown that multiple species of *Pythium* have the capacity to reduce germination of both corn and soybean seed leading to decreased emergence. In addition mefenoxam, azoxystrobin, trifloxystrobin, and captan, as seed treatments, may not be able to inhibit infection by all pathogenic species of *Pythium*.

Introduction

➢Pre and post-emergence damping-off of corn and soybeans has become an increasing concern in Ohio as growers have begun planting earlier in the season.

➢Cool moist climates are ideal for many species of *Pythium* which have been reported to cause seedling diseases of corn and soybeans, and in many cases several species of Pythium have been isolated from the same plant, or location.

➢Previous studies in Ohio have recovered *P. catenulatum, P. irregulare, P. paroecandrum, P. splendens*, and *P. torulosum* from soybean using a baiting procedure[1], and *P. arrhenomanes, P. dissotocum, P. graminicola, P. ultimum,* and *P. torulosum* were isolated from corn[2,4] .

➢Metalaxyl and Mefenoxam have been the most frequently used fungicides for the control of *Pythium* spp., but newer strobilurin fungicides are frequently added to increase the efficacy of the seed treatment

➢*Pythium* spp. have demonstrated varying levels of pathogenicity and sensitivity to metalaxyl within and among species .

➢Stand failures have occurred despite the recent change in commercial seed treatment active ingredients.

➢These results have lead to this current investigation characterizing the *Pythium* spp. causing corn and soybean seed and seedling disease in Ohio.

Objectives

➢**Isolate and identify *Pythium* spp. associated with corn and soybean seed and seedling disease symptoms**

➢**Determine the pathogenicity *in vitro* of each *Pythium* isolate on both corn and soybean seeds**

➢**Determine the level of sensitivity of *Pythium* species to mefenoxam, azoxystrobin, triloxystrobin, and captan**

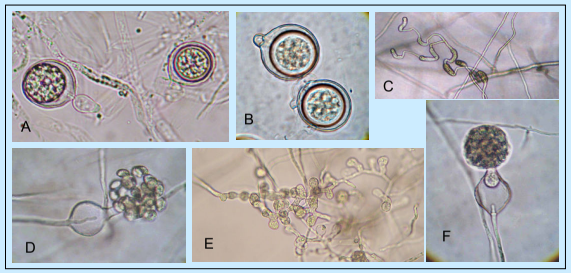

Fig. 1 Reproductive structures of *Pythium* spp. including oogonia and antheridia (A-B), appressoria (C) and globose (D) filamentous (E) and proliferating (F) sporangia

Results & Conclusions

Isolation & Identification

➢105 isolates were recovered from 32 locations. From these isolates,14 species of *Pythium,* as well 2 distinct morphological groups, were identified by morphology and sequence analysis.

➢The phylogeny produced 3 major clades within the genus *Pythium* and a fourth clade for the *Phytophthora* species (Fig 6). All species with globose sporangia were in Clade A, filamentous sporangia were in Clade B, proliferating sporangia in Clade D, and Phytophthora species in Clade C

Pathogenicity

➢All species were more pathogenic on soybean seeds than on corn seeds. Of the 14 *Pythium* speci 6 were moderately to highly pathogenic on corn seeds and 9 were highly pathogenic on soybean seeds. (Fig. 3)

➢This is the first report of *P. inflatum* and *P. attrantheridium* as pathogens of corn and soybean seedlings, and the first report of *P. sylvaticum* as a pathogen of corn seeds and seedlings.

➢These results demonstrate that several phylogenetically diverse species of *Pythium* have the capacity to reduce the germination of corn and soybean seeds

Fungicide Sensitivity

➢There were significant differences ($p<0.01$) in sensitivity to mefenoxam, azoxystrobin, trifloxystrobi and captan both across species (Fig 4) and among isolates of a species

➢Sensitivity to azoxystrobin and mefenoxam were associated with the sporangial morphology of the species. (Fig. 5)

➢It appears that mefenoxam, azoxystrobin, trifloxystrobin, and captan alone are not able to effective control all pathogenic species of *Pythium*, due to varying sensitivities to the fungicides among specie

Materials and Methods

Isolations & Identification

➢Diseased seedling were collected from 40 locations in 20 counties in Ohio and Indiana during the spring of 2005 and 2004.

➢Isolates were identified by morphological characters (Fig 1a-f) using standard keys[3] and by sequence analysis

➢Sequence data were aligned using Clustal-W, and parsimony analysis to create a phylogenetic tree (Fig. 6) was done with Molecular Evolutionary Genetic Analysis (MEGA) version 3.1.

Pathogenicity Assays

➢An *in vitro* pathogenicity assay was utilized to determine the pathogencity of each isolate on LG2540 corn and Sloan soybean seeds

➢The seeds were scored on a 0-3 rating scale (Fig 2) for pathogenicity with 0= 100% germination, 1=70-99% germination, 2= 30-69% germination, and 3= 0-29% germination.

➢Each isolate had 3 replications per experiment and the experiment was repeated 3 times. The entire experiment was carried out for both corn and soybean seeds.

Fungicide Sensitivity

➢Mefenoxam, azoxystrobin, trifloxystrobin, and captan were amended to PCA in a series of dilutions depending on the application rate recommended on the label.

➢Sixty isolates representing all the species and locations were selected to be tested.

➢The diameter of the mycelial growth of each isolate at each concentration was measured at 48, and 72 hours.

➢The diameter of each isolate on the amended media was divided by the growth on the control to calculate the percent inhibition of mycelial growth.

➢The experiment was repeated 3 time.

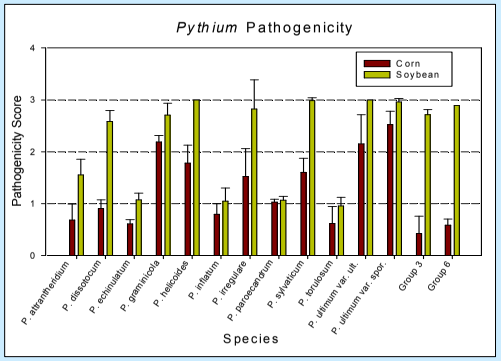

Fig. 3 Pathogenicity of 14 species of *Pythium*. 0-1=low,1-2=moderate, and 2-3=high level of pathogenicity

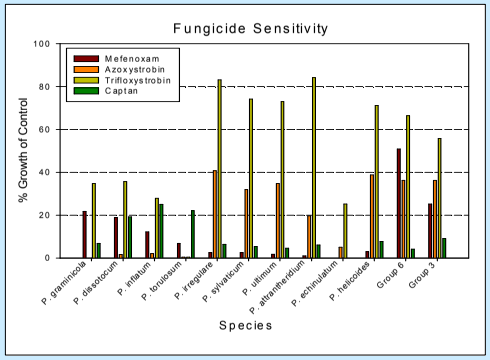

Fig. 4 Sensitivity of 12 *Pythium* species to 4 fungicides. % growth of control is averaged over fungicide concetrations.

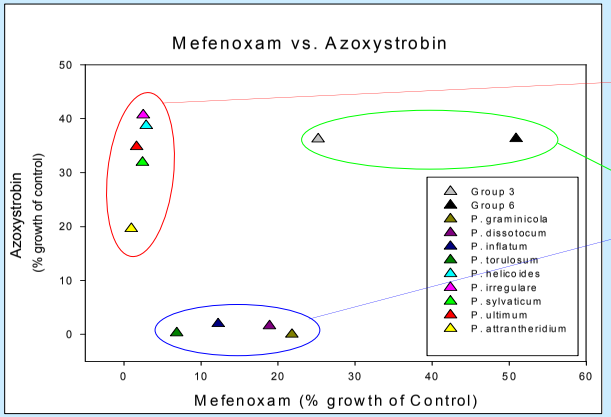

Fig. 5 Sensitivity of *Pythium* species to mefenoxam vs. sensitivity to azoxystrobin Red=globose sporangia, Blue=filamentous sporangia, Green=Proliferating sporangia

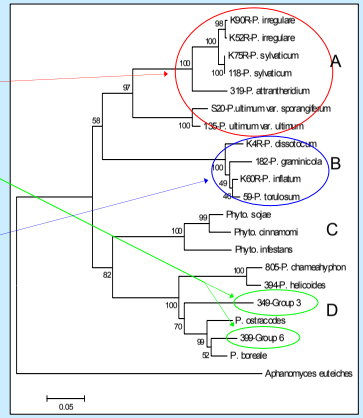

Fig. 6 Consesus tree conferred from ITS1-5.8S-ITS2 region of the rDNA. Bootstrap values for 100 parsimony anylses are given.

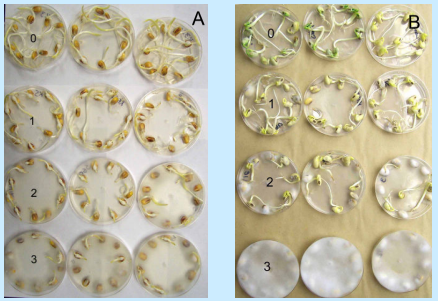

Fig 2. *In vitro* pathogenicity assay with corn (A) and soybean (B) seeds, displaying the 0-3 rating scale based on germination

Literature Cited

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